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Variation in the Composition of the Ruminal Bacterial Microflora during the Adaptation Phase in an Artificial Fermentor (Rusitec)

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ABSTRACT—The RUSITEC system, in its present design, could not maintain microbial populations in conditions comparable to those encountered "*in vivo*". During the adaptatin period, the microfauna and microflora underwent quantitative and qualitative variations (decrease by almost 99% of the ciliate population). Ten bacterial species, which were specifically identified and counted by immunofluorescence, increased during the first two days after the inoculation of the fermentation units. This loss of balance in the indigenous rumen microbial populations suggested that essential interactions occurred between bacteria and between ciliates and bacteria. In RUSITEC at the end of the adaptation phase, as in defaunated animals, propionate productions increased (while acetate productions were reduced) and there were qualitative in the bacterial populations. However, there were differences in the nature of these populations, since in RUSITEC methanogenesis was not inhibited and total VFA production remained stable.

INTRODUCTION

RUSITEC (rumen simulation technique) [6], is a semi-continuous device designed to simulate the functions of the rumen, which is a natural fermentor in the digestive tract of ruminants. *In vivo*, this gastric compartment harbours a large number of anaerobic microorganisms (protozoa, bacteria and fungi) which act in the degradation of the feed ration ingested by the animal. Our understanding of how the fermentative processes become established "*in vitro*" in this kind of fermentor is still incomplete because not enough is known about the behaviour of the different bacterial species. One of the main difficulties in making population counts is a great specific diversity of the groups of bacteria.

So, in this work, we have studies 10 bacterial species with enzymatic abilities representative of the ruminal microflora, and we have followed their evolution by indirect immunofluorescence during the crucial period of stabilization of the fermentative processes, which corresponds to the adaptation phase of the artificial system.

MATERIALS AND METHODS

We have used the RUSITEC fermentation system as previously described by Czerkawski and Breckenridge [6]. Ground lucern hay (15g dry matter) was placed in 8×13 cm nylon bags having a 250 μm porosity. The characteristics of the substrate have been detailed elsewhere [13]. The two fermentation units used, fermentor I and II, were perfused with a solution of artificial saliva, prepared according to Mc Dougall's formula [19] with a delivery rate of 0.03 h-1. The experiment was made during the adaptation phase of the fermentors, the first week after the initial inoculation (days 1 to 5). Microbial counts and analysis of the fermentative parameters were made in the "*sensu stricto*" liquid phase of the reactors [21] at different times before (T0) and after introduction of the lucern hay (T1.5h, T3h, T5h, T7h, T10h, T14h, T19h and T24h=T0 of the following day). In this work, we have chosen to analyse only the liquid phase, because the sampling times were too close to allow a good regeneration of the fermentative conditions if we had taken samples on the solid and the liquid-associated-to-the-substrate phases (these samplings need an opening of the fermentation units). A further publication will compare the microbial populations and the fermentative parameters between the three phases.

Inoculation and running of the system

The two fermentors were intially inoculated with both a liquid and a solid fraction sampled from fasted sheep fitted with rumen canula [6]. The animals were fed with lucern and had conventional flora and fauna harbouring the 4, 5 or more common genera of protozoa (*Isotricha, Dasytricha, Epidinium, Eudiplodinium* and *Entodinium*). The rumen fluid was filtered at 39°C first on a metal grid (mesh 1.5 mm) and then on 2 layers of gauze. Five hundred ml of this homogenised filtrate were poured into each reactor followed by the same volume of a mixture of artificial saliva (300 ml) and distilled water (200 ml), adjusted to pH 7. The solid phase, which was picked up on the grid after filtration, was used to fill the bags called "initial inoculation bags" (one bag of about 50 g by reactor), providing half-digested matter and a good variety of microorganisms, attached to the plant particles. A single bag of 15 g of lucern hay was also placed in each fermentor. Throughout the experimental period, the RUSITEC was run according to the protocol of Czerkawski and Breckenridge [6].

Microbial counts

At each sampling, the ciliate protozoa were fixed by a lugol

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solution, in Dolfuss jars and counts were made immediately afterwards [22]. The bacterial samples (1 ml of "fermentor fluid" fixed with 1 ml of neutralized formalin at 4% in PBS buffer) were kept at 4°C. Indirect fluorescence counts of total bacteria were made after staining with acridine orange (AO), which is a commonly used method [2, 7, 9] and which has been adapted to the rumen ecosystem [21]. The staining of bacteria was made by mixing 1 volume of the diluate sample with 1 volume of an acridine orange solution at 2% (w/v); after 10 min, this solution was filtrated on a polycarbonate filter (Irgalan blue Nuclépore DMF 25 mm, porosity 0.2 μm) using a vacuum system. Some drops of Triton X-100 were put before filtration on the filter, to minimize the ground fluorescence and to obtain a more homogeneous distribution of the bacteria. The filters were then put on a slide and were observed under an epifluorescence microscope (×575).

For immunofluorescence counts, specific antibodies were raised against the 10 species having fermentative, hydrolytic and/or methanogenic activities representative of rumen bacteria (see Table 1). All strains were obtained from INRA collection (Zootechnical Center of Clermont-Theix, France). The antibodies (A.B.) were prepared in our laboratory with pure strains injected in white New Zealand rabbits, according to the technique of Conway de Macario et al. [4]. A first series of experiments showed that no cross-reactions could be detected between the different species of bacteria with our A.B., and that their S titre varied between 1/400 and 1/800 and their T titre between 1/32 000 and 1/64 000. The primary A.B. were used at a 1/100 concentration with an incubation of 30 min in a damp chamber at 39°C. They were then visualized by binding with a goat-anti-rabbit IgG conjugate (FITC) with a concentration of 1/100 in the same conditions as for primary A.B. Bacterial counts were made on slides and were observed under an epifluorescence microscope (×575).

We have based our counting technique on that of Ogimoto [20], i.e. we have counted only some fields of a known area and then we have estimated the total bacterial concentration. For that, we have also determined the surface of each "dried drop" of bacterial deposit, which was an ellipse; the two diameters were measured under microscope (×575) with a squared reticle (10×10), which was also used as counting field. To minimize the heterogeneity of bacterial distribution of the deposit, we have used a low dilution rate for samples ($2.5\ 10^{-2}$), with a drop volume of 2 μl. As we have considered that the average concentration of total bacteria in the rumen fluid was 10^{10} cells/ml (see results), the deposit volume was equivalent to 5.10^5 bacteria/drop.

Microscopic examination has confirmed the low heterogeneity in cell distribution. We have counted x fields having an area of s μm^2 (the fields were selected both at the periphery of the drop and near the center), and so we have obtained ($m \pm \sigma$) bacteria counted in a single field. Using the formula for total bacterial counts after AO staining [21], we have obtained:

$$\frac{mS\ 10^6}{sv} \pm \frac{\sigma S\ 10^6}{sv} \text{ cells/ml}$$

where $S = \pi DI/4$ (D and I being the 2 diameters of the elliptic drop) and v the volume of the sample including the dilution rate ($v = 5.10^{-5}$ ml). Generally, and because of the quick fading of fluorescence, we used to count x = 5 fields.

To calculate the percentage of the 10 bacterial species counted by immunofluorescence in relation to the total population (stained by AO), we have determined the cell concentration of some pure cultures with both methods and we have compared the results to determine a corresponding factor. The Table 2 gives example of some results obtained with two pure cultures of bacteria. We have checked that the difference between the two methods was almost constant whatever can be the strain, but depended of the worker. Here, the same experimenter had counted the bacteria.

Fermentative parameters

At each sampling, the pH and the redox potential were measured with a Schott-Gerate CG 817-T apparatus equipped with a 1042 A (pH) or a Pt 42A (mV) electrode. Ammonia nitrogen ($N\text{-}NH_3$) content was measured according to the method of Weaterburn [23]. The analysis of short-chain organic acids (volatile fatty acids = VFA) was made in a gaseous phase chromatograph fitted with a flame ionization detector [15]. The gas mixture produced was also analy-

Table 1. Morphological and metabolic specifications of the 10 bacterial species belonging to the AB flora (see text)

Bacteria	Strains	Form	Gram	Ferm. subst.	Gas	Products
S. bov	FD10	C	+	S, Ce, P, SS		L (A, F)
R. fla	007	C	+	C, Ce	CO_2, H_2	A, Sc (F, L)
R. alb	7	C	+	C, Ce	CO_2, H_2	A, E (F, L)
E. cel	C	R	+	C, Ce		A, B, F, L, Sc (Pr)
E. lim	20543	R	+	Ce	CO_2, H_2	
M. rum	Mr	R	+	H	CH_4	
F. suc	S 85	R	−	S, C, Ce		A, Sc
S. rum	W	R	−	S, GI, L, P, SS	H_2S	A, L, Pr (Sc)
L. mul	LM	R	V	S, Ce, P, Pe	CO_2, H_2	A, E, F, L, Sc
B. fib	D1	R	V	S, C, P, SS	CO_2, H_2, H_2S	F (A, B)

S. bov: Streptococcus bovis
R. alb.: Ruminococcus albus
E. lim: Eubacterium limosum
F suc: Fibrobacter succinogenes
L. mul: Lachnospira multiparus
R. fla: Ruminococcus flavefasciens
E. cel: Eubacterium cellulosolvens
M. rum: Methanobrevibacter ruminantium
S. rum: Selenomonas ruminantium
B. fib: Butyrivibrio fibrisolvens

C: cocci; R: rod; +, − and V: positive, negative and variable Gram staining; Ferm. subst.: fermented substrates; Ce: cellobiose; C: cellulose; Gl: glucose; H: hydrogen; L: lactate; P: proteins; Pe: pectines; S: starch; SS: soluble sugars; Products: A: acetate; B: butyrate; E: ethanol; F: formatc; L: lactatc; Pr: propionate; Sc: succinate.

TABLE 2. Comparison between the 2 numbering methods (× 10^9 cells/ml)

Bacteria	S	AB	AO
R. flavefasciens	1	4.27	7.12
	2	3.55	6.46
	m	3.91±0.55	6.79±0.47
	D	−42.4%	100%
F. succinogenes	1	3.12	5.30
	2	2.86	4.44
	m	2.99±0.18	4.87±0.61
	D	−38.6%	100%
	M	−40.5%	100%

AB: immunological counts (antibodies): AO: counts by Acridine Orange; D: difference in % between the 2 methods for a specific bacterial strain; m: average value for 2 samples (S1 and S2) by a given method; M: total average percentage of dissapearance.

sed by gas-chromatography at 80°C (Girdel 30 apparatus) [15].

RESULTS

Ciliates

During the adaptation phase, the ciliates population decreased by almost 99% falling from 10^5 cells/ml after inoculation (D1, T0) to 10^3 cells/ml at D5 T24h (Figure 1A). Slight variations in the total number of ciliates were observed in the liquid phase, but there was no regular daily fluctuation. This overall pattern involved all the present ciliates: *Entodinium, Isotricha, Dasytricha, Epidinium* and *Eudiplodinium*. This last genus disappeared entirely from the liquid phase after 4 days of running (Figure 1B). The decrease in numbers was observed in both fermentation units and was greatest during the first 48 hours: the total ciliate density was 93% lower than the start density by the beginning of the third day (D3, T0) (Figure 1A). At the end of the experimental period, only the ciliates belonging to the genus *Entodinium* were present in large numbers.

Bacteria

Bacterial counts on pure culture obtained with the immunofluorescence method which used specific antibodies were 40% lower than those yielded by epifluorescence after AO staining (see Table 2). This lower result of AB staining, compared with the AO one, probably came from the washings made to eliminate the AB-FITC solution. In all the following results, the values concerning the 10 bacterial species have been corrected by the corresponding factor. Although less marked than in the ciliates, the decrease in the total number of bacteria reached an average of 70% during

FIG. 1A. Evolution of the total ciliate population during the adaptation period (D1 to D6: example, fermentation unit 1).

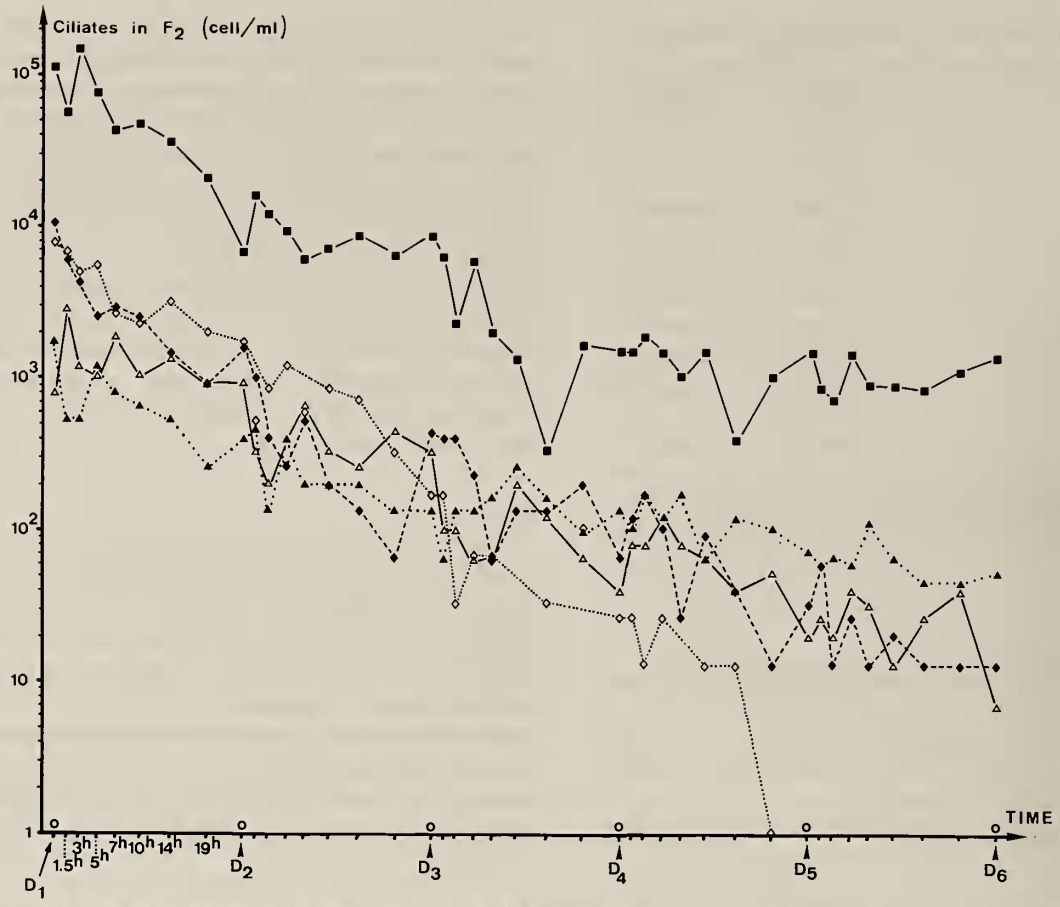

FIG. 1B. Evolution of the different ciliate populations during the adaptation period (D1 to D6: example, fermentation unit 2). △: *Isotricha*; ▲ : *Dasytricha*; ◆: *Epidinium*; ◇: *Eudiplodinium*; ■: *Entodinium*.

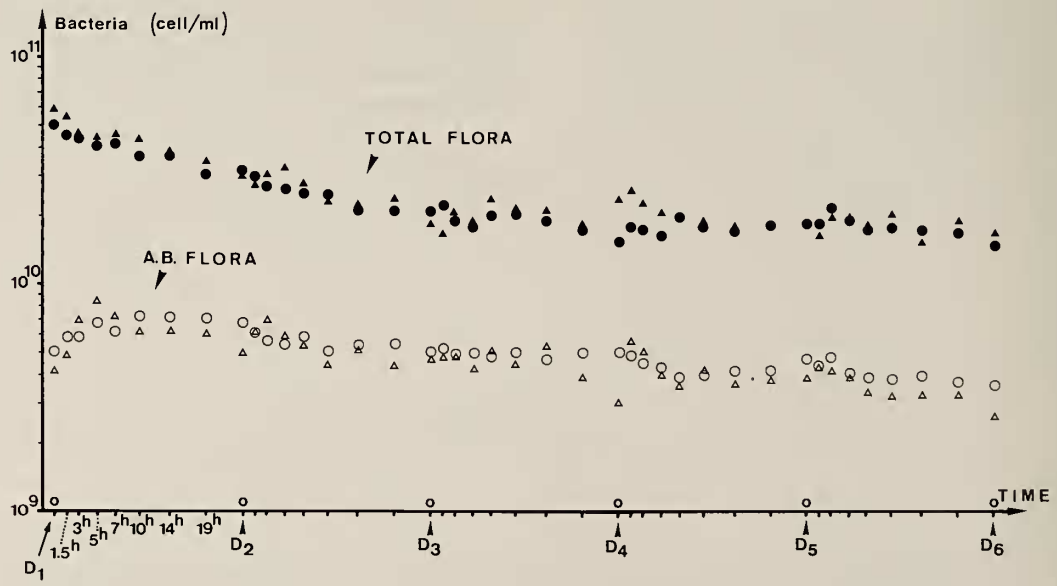

FIG. 2. Evolution of the total bacterial population (black symbols) and of the AB flora (see text) (while symbols) in the 2 fermentors. F1: triangles; F2: circles.

the adaptation phase of RUSITEC (Figure 2), and, as in the former population, the decrease was greatest during the 2 first days (−58% in one fermentation unit and −69% in the other).

The predominant type of bacteria was cocci, which were observed throughout the experimental period at an almost constant level of 86% (Table 3). Bacteria staining by AO appear red or green, depending on the cellular concentration

TABLE 3. Average percentage of cocci during the different experimental days (D) in the 2 fermentation units

Day	F1	F2
1	86.2±0.9	86.1±1.1
2	86.8±1.5	86.0±1.7
3	86.4±1.9	86.3±3.2
4	85.6±2.1	84.9±3.3
5	86.5±1.6	86.0±2.3

of nucleic acids (DNA and RNA): the red bacteria (full of RNA) are considered as the most physiologically active cells, and the green ones (where the RNA concentration is too low to hide the green fluorescence of DNA) are considered as the less active cells [21]. These results are related to the ATP, ADP and AMP levels [21].

In our experiment, the percentage of red bacteria varied 72 to 66% in one fermentor and from 73 to 66% in the other, during the adaptation period, and in particular during the first 2 days after inoculation (Table 4). The total bacterial concentration was equivalent to the sum of red and green bacteria. The 10 bacterial species selected for counts represented 7 to 10% of the initial inoculum (=total inoculum) (Table 5). Unlike in the overall population (total flora), the numbers of bacteria recognised by their specific antibodies (AB flora) increased during the first 2 days (Figure 2), by 40 to 42% depending of the fermentation unit (Table 6). Of the 10 species which were immunologically recognised, the most abundant were *Ruminococcus albus, R. flavefasciens, Methanobrevibacter ruminantium* and *Fibrobacter succinogenes*, and the less abundant *Butyrivibrio fibrisolvens* and *Lachnospira multiparus* (Table 5). The statistical comparisons (Student's test) made between the daily average percentages of each of the 10 species showed that their distribution range was not modified during the adaptation period (Table 6).

TABLE 4. Daily average values (%) of red bacteria (after AO staining) in the 2 fermentors

Day	F1	test	F2	test
1	73.4±2.7	1–2	71.7±1.3	1–2#
2	70.5±2.4	2–3#	69.2±1.4	2–3
3	65.4±2.6	3–4	66.9±2.8	3–4
4	67.2±3.1	4–5	65.3±2.2	4–5
5	67.6±3.6	1–5#	65.8±1.8	1–5#
		2–5		2–5#

#: significant difference (Student test, α=5%). We have: 100% of bacteria=red bacteria+green bacteria.

TABLE 5. Density of specifically labelled bacterial populations ($\times 10^8$ cells/ml) and percentages in comparison with the total bacterial population (AO counts) (day 1, T0)

Bacteria	F1		F2	
	numbers	%	numbers	%
R. alb	6.05±0.91	1.03±0.16	7.74±0.92	1.54±0.18
R. fla	5.91±1.29	1.01±0.22	7.59±0.41	1.51±0.08
F. suc	3.68±0.77	0.63±0.13	4.91±1.12	0.98±0.22
S. bov	3.74±0.84	0.64±0.14	3.98±0.39	0.79±0.08
M. rum	2.81±0.29	0.48±0.05	6.26±0.43	1.25±0.09
S. rum	5.10±0.85	0.87±0.14	4.48±0.74	0.89±0.15
E. cel	3.61±1.08	0.61±0.18	6.43±0.42	1.28±0.08
E. lim	5.04±1.20	0.86±0.20	3.64±0.25	0.73±0.05
B. fib	3.82±0.83	0.65±0.14	3.44±0.22	0.69±0.04
L. mul	2.17±0.63	0.37±0.11	2.69±0.24	0.54±0.05
Total	41.93±8.69	7.15±1.47	51.16±5.14	10.20±1.02

TABLE 6. Statistical comparisons (Student test: α=5%) of the daily average concentrations of the 10 specifically labelled bacteria (AB flora): days 1 to 5 (D1 to D5)

Bacteria	D1-D2	D2-D3	D3-D4	D4-D5	D1-D5	D2-D5
R. alb	*+		°−		°+	°+
R. fla	*+		*−		*+	°+
F. suc	°+	°+	°−		*+	
S. bov	*+	°+	°−		*+	
M. rum	*+	°+			*+	
S. rum	°+	°+			°+	
E. cel	°+	°+			°+	
E. lim	°+	*+			*+	°+
B. fib		°+			*+	
L. mul	*+	°+	°−		*+	°+

*: significant difference in the 2 fermentation units; °: significant difference in a single fermentation unit; + and −: general evolution.

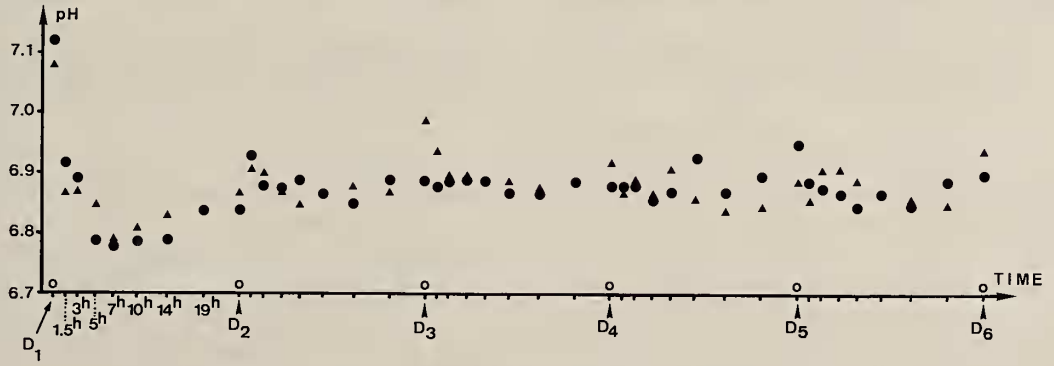

FIG. 3. pH evolution in the 2 fermentation units. F1: triangles; F2: circles.

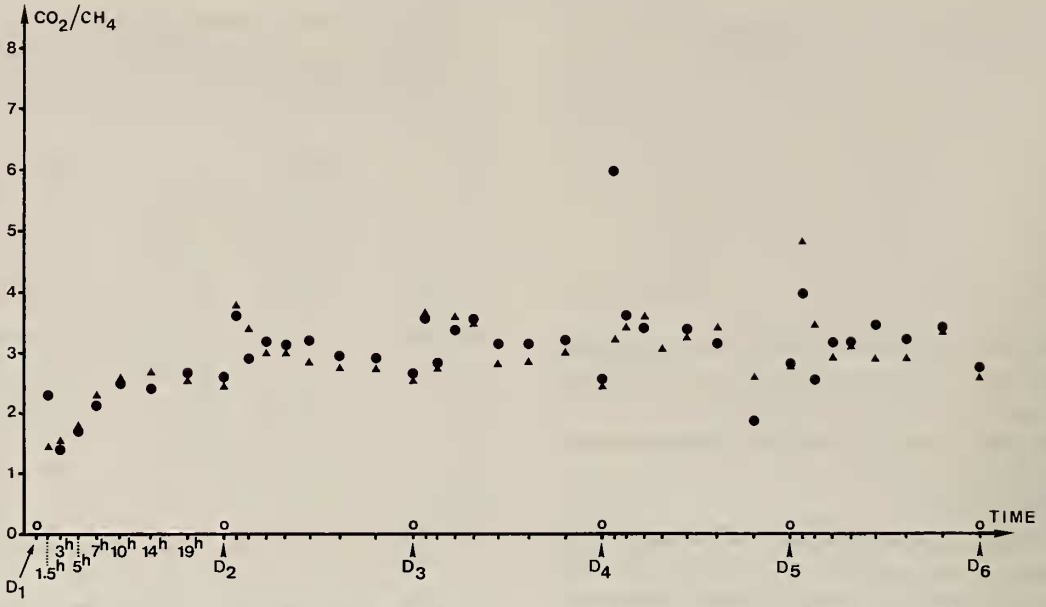

FIG. 4. Evolution of the CO_2/CH_4 ratio in the 2 fermentation units. F1: triangles; F2: circles.

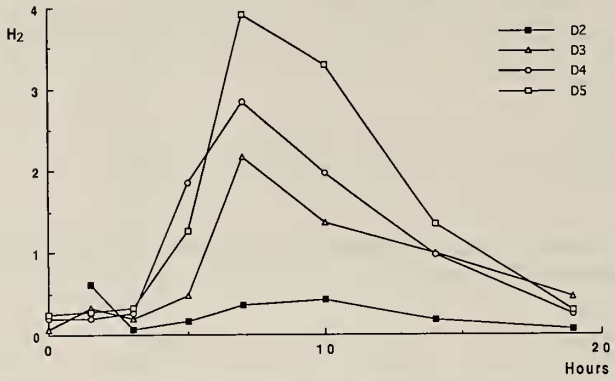

FIG. 5. Evolution of hydrogen concentration (% in the gas mixture) during the adaptation period: days 2 to 5 (D2 to D5) (example: fermentation unit 1).

Fermentative parameters

The fermentative profile obtained during the adaptation period was satisfactory in spite of the decrease in the microbial populations since, at the end, the remaining bacteria were able to maintain the fermentation process. The redox potential varied between −320 and −360 mV and the pH between 6.8 and 7.0 (Figure 3), values which were compatible with the survival of the microorganisms. Gas production showed very little variations during the experimental period. The high proportion of CO_2 in the gas mixture produced (73–78%) shows that anaerobic conditions in the fermentors were well sustained. The proportion of methane ranged from 20 to 30%: it was greatest at T0, decreased at T1.5h and rose again sharply at T3h. The CO_2/CH_4 ratios varied between 2 and 4, and were highest at T1.5h (Figure 4). Hydrogen production followed a regular daily pattern: peak concentrations occurred between T7h and T10h, and were higher at the end of the experimental period (D4, D5) (Figure 5).

Apart from the fluctuations observed on D1, there was a gradual decrease in N-NH3 production (Table 7) during the adaptation phase, while that of the volatile fatty acids remained fairly stable at around 72–76 mM/l (Table 7). Analysis of the percentage of each VFA produced in the mixture showed a change in the fermentation profile during the adaptation period in both fermentors (Tables 8 and 9). The proportion of acetic and butyric acids decreased, from 70 to 65.5% and 1.2 to 0.7% respectively, while that of propionic, butyric valeric and caproic acids increased, from 18 to 20%, 7.3 to 9.4%, 1.5 to 2.0% and 0.15 to 0.40% respective-

TABLE 7. Daily average values of ammonia (mg/l) and VFA (mM/l) productions in the 2 fermentation units (F1 and F2)

Day	NH^3				VFA			
	F1	test	F2	test	F1	test	F2	test
1	106.3	1–2#	110.9	1–2#	52.33	1–2#	58.84	1–2#
2	150.6	2–3#	140.3	2–3	74.19	2–3	72.25	2–3
3	115.4	3–4#	143.1	3–4#	75.79	3–4	73.66	3–4
4	90.0	4–5	109.7	4–5	76.22	4–5	71.24	4–5
5	83.8	1–5	90.3	1–5	77.41	1–5#	74.36	1–5#
		2–5#		2–5#		2–5		2–5

Statistical comparisons (Student test: α=5%). #: significant difference.

TABLE 8. Daily average percentages of the different VFAs produced (example: fermentation unit 1)

Day	C2	C3	IC4	C4	C5	C6
1	69.60±0.80	18.56±0.16	1.17±0.07	7.28±0.44	1.45±0.16	0.15±0.01
2	68.54±0.71	19.11±0.32	1.08±0.08	7.66±0.47	1.57±0.05	0.17±0.02
3	68.17±0.39	19.62±0.25	0.85±0.09	7.96±0.41	1.59±0.05	0.21±0.01
4	66.69±0.86	19.94±0.49	0.71±0.09	8.86±0.42	1.82±0.11	0.30±0.03
5	65.56±0.93	20.03±0.28	0.72±0.05	9.38±0.67	1.98±0.06	0.41±0.03

C2: acetic acid; C3: propionic acid; IC4: isobutyric acid; C4: butyric acid; IC5: isovaleric acid; C5: valeric acid; C6: caproic acid.

TABLE 9. Statistical comparisons (Student test: $\alpha=5\%$) of the daily percentages of each VFA produced in the mixture: days 1 to 5 (*D1 to D5*)

VFA	D1-D2	D2-D3	D3-D4	D4-D5	D1-D5	D2-D5
C2	*−	°−	*−	°−	*−	*−
C3	*+	*+			*+	*+
IC4	*−	*−	°−		*−	*−
C4	°+		*+		*+	*+
IC5			*−		*+	
C5		°+	*+	*+	*+	*+
C6	*+	*+	*+	*+	*+	*+

*: significant difference in the 2 fermentation units; °: significant difference in a single fermentation unit; + and −: general evolution.

ly. The proportion of isovaleric acid was at a stable level of 1.7% on average (Table 8).

DISCUSSION

Microorganisms

The considerable decrease in the ciliate and bacterial populations (−99% and −70% respectively) did not result in correspondingly adverse effects on the overall fermentation profile: VFA production during the adaptation phase remained high (72–76 mM/l), as did the production of methane (25–30%) and ammonia (higher than 50 mg/l), in spite of the fact that dilution rate of the liquid phase was 0.03h-1. At the end of the experimental period (from D3 to D5, T24H), The remaining bacterial species-like those of the AB flora-seemed to possess the multienzymatic abilities to achieve successfully the fermentation. There are various possible reasons for the decrease in the ciliate and bacterial populations, related to both the design and the running of RUSITEC: presentation of the substrate, mixing system, delivery and chemical composition of the artificial saliva, outflow of the liquid phase and microorganisms.

In contrast, pH values did not seem to affect the microbial populations and, like the values of the redox potential, were always compatible with the survival of the cells. It is also possible than the decrease in cell number of Protozoa was due partly to modification of essential interactions between ciliates and bacteria. Various authors have shown that ciliates can be selective predators for bacteria [1, 3, 8, 24]. The absence of certain bacterial species, as the result of the *in vivo-in vitro* transition, could therefore have a direct effect on one or several ciliate genera. Likewise, in the symbiotic-type relations between bacteria [5] or between ciliates and bacteria, the cell deficit in some bacterial populations, or a metabolic deficiency in the fermentor, could affect the development and the maintenance of the other populations [11, 12].

The evolution of the AB flora showed that during the adaptation period of RUSITEC there was a qualitative change in the bacterial populations, since the total and relative percentages of the bacterial species which were specifically recognized increased during the first 2 days of the experimental period. There are two possible explanations for this change: 1) there was a selection in the AB flora of populations that were better adapted to the culture conditions of RUSITEC; 2) there was a decrease in the number of other bacteria (that were not counted by AB but only by AO), because they were more "sensitive" to the same conditions. These two possibilities should be taken into account since it is likely that the strains of the AB flora are among the most easily isolated bacteria and therefore the less sensitive to the changes in physiological conditions.

The increase in the number of bacteria in the AB flora was accompanied by a decrease in that of the ciliates populations, and these two facts may be correlated. However, the decrease in ciliate numbers cannot be positively attributed to some variation in the AB flora. It would be of interest to do further studies using other immunological markers. The decrease in the percentage of red bacteria (which are the most physiologically active cells) was observed mainly during the first two days, which lends weight to the hypothesis that a certain microbial "stress" occurred when the fermentors were inoculated. This fact was correlated to the increase of the percentage of green bacteria, less active than the red ones (as we have total bacteria=red+green bacteria).

Fermentative parameters

In the RUSITEC content at the end of the experimental period, there was a variation in the composition of the VFA produced, similar to that observed in comparisons of the faunated and unfaunated states *in vivo* [16]. In particular there is a reduction of acetate production which could be due in part to the fact that ciliates producing acetic acid during carbohydrate fermentation [10] were only present in very small numbers at the end of the experimental period. This

reduction leads to an increase of the relative percentage of propionate which could also be the reflect of non optimal conditions for methanogenesis. Hence, since propionic acid has an inhibitory effect on protozoa [18], a rise in its proportion could increase the extent of disappearance of the ciliates.

The metabolic pattern of VFA formation in RUSITEC could also come from a shift in production from acetyl-coA to butyrate, which would decrease the production of acetate, as observed in the rumen of defaunated animals receiving a diet rich in carbohydrates [16]. Because the ciliates populations were smaller during the second half of the experimental period, they had less effect on proteolysis, and this may have contributed to the decrease in total ammonia production observed in the contents of fermentors. As the amount of amino-acids produced by the degradation of proteins and alimentary peptides decreased, the production of ammonia from these metabolites was also lower.

Fermentation in the RUSITEC system at the end of the adaptation period is similar to that in defaunated animals, and in both cases there are qualitative variations in the composition of the bacterial populations [13, 15]. However the composition of the bacterial populations is not probably the same since, unlike in defaunated animals [12], the total VFA production in RUSITEC did not decrease during the experimental period and the methanogenesis was not inhibited.

CONCLUSION

The RUSITEC system, as designed by Czerkawski and Breckenridge in 1977, is an interesting technique fot studying fermentation "*in vitro*", but can only simulate "*in vivo*" defaunated states. In its present form, the RUSITEC system cannot reproduce the "*in vivo*" state of conventionally reared animals: ciliate populations are eliminated in the fermentor and probably many bacterial species too, and so certain parameters need to be studied and perhaps modified.

Modifications could be made on the physical parameters (such as variations in the rate of artificial saliva, changes in the form of substrate, addition of a solid matrix to increase the retention of the ciliates) on the chemical parameters (such as a second inoculation with new microorganisms at the end of the adaptation period).

The desappearance of ciliates in RUSITEC is due to a loss of balance in the bacterial populations. Recognizing one bacterial species amongst all the others in the rumen is still a difficult problem to surmount. However, techniques using immunological markers to recognize bacteria may lead to a better understanding of the intricate microbial interactions that occur between ciliates and bacteria in the rumen ecosystem.

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